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NUTRITION REVIEWS

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NUTRITION REVIEWS

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TRACE ELEMENTS IN BIOCHEMISTRY AND NUTRITION

Oligo-elements, trace elements, micronutrients, microelements, rare elements, and minor elements are terms which have been applied to a group of elements occurring in biologic systems in such small concentrations that they have usually been found present qualitatively but not measured quantitatively. Among this group the following are generally included: aluminum, antimony, arsenic, barium, boron, bromine, cadmium, chromium, cobalt, copper, fluorine, gallium, iodine, iron, lead, lithium, magnesium, manganese, mercury, molybdenum, nickel, rubidium, scandium, silver, strontium, tin, titanium, vanadium, and zinc. These elements have been grouped together quite arbitrarily. They have in common (1) the difficulty of measurement because of low concentration, which varies from 1×10^{-6} to less than 1×10^{-12} g. per gram of wet tissue, and (2) a resistance to definitive appraisal as to physiologic function. The terms "oligo-element" or "oligo-metal" (oligo meaning scanty (Greek)), have been used in the French literature for some time and are suggested as less confusing than some of the other designations which have additional connotations or prejudicial implications.

The evolution of knowledge in this field has been slow. Most of the information has been accumulated in the last twenty-five years, though interest in many aspects of the problem dates back at least a century. The presence of copper in the oxygen-transporting hemocyanin of mollusks was first recognized more than a century ago (E. Harless, *Müller's Arch. f. Anat., Physiol., p. 148 (1847)*). Studies on hydroponics by Sachs (1860) and Knop (1861) provided a concrete demonstration that plants require trace elements for growth, while J. Raulin's work with *Aspergillus niger* (*Ann. sci. nat. bot. 5e sér.*, **11,** *93 (1869)*) extended this concept to fungi. E. Baumann's classic discovery of iodine in the thyroid (*Z. f. physiol. chem.* **21,** *319 (1895–96)*) pointed to the essential role of an element of high atomic weight in the control of mammalian metabolism. Iodine had been used empirically seventy-five years earlier as an efficient remedy for goiter (J. R. Coindet, *Ann. d. chim. phys.* **15,** *49 (1820)*). C. Bernard's investigations on cell respiration ("Leçons sur les effets des substances toxiques et médicamenteuses," *Bailliere et fils, Paris (1857)*), those of C. A. MacMunn on cytochrome (*Phil. Tr. Roy. Soc. (London)* **177,** *267 (1885)*), and the studies of W. Spitzer on iron and oxidative processes (*Pflugers Arch. f. d. ges. Physiol.* **67,** *615 (1897)*) gave first suggestions which eventually led to the discovery of metalloenzymes (enzymes containing a metal as an indispensable part of the molecule) and of metal/enzyme interactions in catalysis. These investigations of the biochemical role of the oligo-elements have been carried forward by Warburg, Keilin, Theorell, Sumner, Nelson, Dawson and their associates. Hart, McCollum, Steinberg, Elvehjem and their students have greatly enhanced our understanding of the role of these elements in the nutrition of microorganisms, higher plants, and animals.

Oligo-metal deficiency states in nature (B. Sjollema, *Biochem. Z.* **267,** *151 (1933)*) were emphasized by the discovery in Holland of severe copper deficiency diseases in plants and in animals using these deficient plants as fodder. This was soon followed by the discovery of cobalt deficiencies in sheep in Australia (J. F. Filmer and E. J. Underwood, *Australian Vet. J.* **10,** *83 (1934)*; H. R. Marston, *Australian Council Sci. Indust. Res. J.* **8,** *111 (1935)*).

The effectiveness of metals in such minute quantities, their interaction with enzymes, and the profound metabolic changes resulting from deficiency, intoxication, and imbalance all suggest an analogy to the vitamins. Though such reasoning by analogy is not convincing, the recent identification of cobalt as an integral part of a vitamin has provided a link between the metals and the vitamins. Previous to the investigations on vitamin B_{12}, when cobalt was demonstrated in living systems, the presence of this element was often considered to be accidental. These studies identify one of the trace elements as a part of a vitamin playing a cardinal role in the nutrition of man and many other forms of life.

The biophysical and biochemical study of these elements has not been systematic. There is a general lack of quantitative chemical data in normal and pathologic states. Even when such data are obtained, accuracy is often wanting because of the use of inadequate methodology. In all of such work valid criteria of biologic effectiveness have been difficult to establish. Deprivation, intoxication, or balance studies on plants and animals have usually been aimed at the production or prevention of abnormal states identified by symptomatology and/or pathology. Because these elements appear to be biologically active in concentrations of less than one millionth of a gram per gram of biologic substance, these types of experiments have not always proven as helpful as they have in other fields of nutritional research. The choice of a rigidly controlled chemical environment, appropriate dietary intake, and good experimental material is even more critical for the success of such experiments than usual. One often faces the danger that a given element may be dismissed from further consideration because an inadequately planned experiment yielded negative results. Such findings are accepted uncritically because they may coincide with the prevailing opinion of the day, or it is presumed that a deficiency state would be unlikely to occur in nature because of the element's widespread occurrence. Even when positive results are obtained, the actions of different metals may be interrelated and may be much more complex than a casual perusal of the data on any one element would indicate. The possibility of imbalance between metals, rather than outright deficiencies or intoxications caused by them, must be borne in mind. A few examples may illustrate the point. The adverse response of rats to high zinc diets is reversed by copper, iron, and liver supplements (S. E. Smith and E. J. Larson, *J. Biol. Chem.* **163,** *29 (1946)*). The presence of abnormally high concentrations of molybdenum in forage causes what is known as molybdenosis in cattle; this process is reversed completely by copper supplements (W. S. Ferguson, *J. Agr. Sci.* **33,** *116 (1943)*). An increased copper requirement in the presence of high concentrations of molybdenum has been demonstrated (G. K. Davis, *in* "A Symposium on Copper Metabolism," edited by W. D. McElroy and H. B. Glass, *The Johns Hopkins Press, Baltimore (1950)*). The toxicity of nickel, cobalt, cadmium, zinc, and manganese to *Escherichia coli* is lowered significantly by increasing concentrations of magnesium in the culture medium, and the amounts of nickel and cobalt actually bound by the cell are markedly decreased (P. H. Abelson and E. Aldous, *J. Bact.* **60,** *401 (1950)*). A series of papers by R. A. MacLeod and E. E. Snell (*Ibid.* **59,** *783 (1950)*; *J. Biol. Chem.* **170,** *351 (1947)*) strongly support the concept of antagonisms between metals.

Valid criteria for the appraisal of biologic effectiveness of oligo-elements must be established, if some of the vagaries of the investigative efforts are to be removed and their scope is to be extended. Objective criteria for the determination of the role of oligo-metals have been sought by analytic chemical and physical methods, by nutritional studies, and by studies of intermediary metabolism.

Relatively little work has been devoted

to the establishment of normal levels of the elements for a particular system. Through advances in chemical and physical knowledge during the past decade, great strides have been made in the design of applicable instruments and methods permitting the determination of minute concentrations of elements with precision. Because of these developments, copper, iron, magnesium, and zinc can now be measured quite accurately to permit a better understanding of their physiologic function and a more exact distinction of their individual stature, in a sense removing them from the category of "trace" constituents. In the cases of iodine and fluorine, their physiologic functions have long been suspected and have provided stimuli to the development of adequate technics for their chemical appraisal.

Microchemistry (E. B. Sandell, "Colorimetric Determination of Traces of Metals," *2nd edition, Interscience Publishers, New York (1950)*), polarography (I. M. Kolthoff and J. J. Lingane, "Polarography," *Interscience Publishers, New York (1946)*), and emission spectrography (G. R. Harrison, R. C. Lord, and J. R. Loofbourow, "Practical Spectroscopy," *Prentice Hall, Inc., New York (1948)*) have been advanced to a point where determinations of oligo-metals may be accepted with greater certainty than would have been advisable twenty years ago. Of these technics emission spectrography is peculiarly suitable. It permits the simultaneous, quantitative assessment of some 20 to 30 elements of biologic interest occurring in concentrations varying from 1×10^{-6} to 1×10^{-9} g. per gram of wet tissue. For individual elements absolute sensitivities of 1×10^{-12} g. per gram of tissue may be reached in amounts of sample containing less than 10 mg. of ash. It thus becomes possible to ascertain the *patterns* of distribution of these elements in normal systems and their deviations in pathologic ones. The coincident use of isotope studies would add further physiologic significance to such data by elucidation of the dynamics of the systems investigated. The development of yet more sensitive tools presently under way offers hope of measuring even lower levels of concentrations of the oligo-elements.

Since oligo-elements have most frequently been found associated with enzyme systems, the efforts of most workers have centered about activity determinations and physical-chemical characterization of metallo-enzymes. Because of their ability to combine with metals, such reagents as cyanide, azide, sulfide, and carbon monoxide, as well as many chelating agents such as 8-hydroxyquinoline, have served an important function in distinguishing metallo-enzymes and inhibiting their action.

In other studies trace metals are added to a more or less purified enzyme system of known activity. The further activation or inactivation thus produced is often tacitly considered to indicate the biologic effectiveness of the element under investigation, even though different and chemically unrelated elements may substitute for one another. This type of metal/enzyme interaction may be contrasted with that of a separate, small group of metallo-enzymes, each of which contains one specific element as an irreplaceable component of the molecule (*see* B. L. Vallee, *Scientific Monthly* **72,** *368 (1951)*). If an added oligo-metal produces an increase in activity, this is often assumed to be evidence that the conditions under which the activation occurs are physiologic. Though this mode of approach has been most informative and the reasoning may yet be accepted in some cases, no substantiating proof that it is a reliable procedure has thus far been adduced. Arginase, for example, has been found to be activated by manganese, vanadium, iron, cadmium, nickel, and cobalt in the test tube. Whether this implies that any or all of these elements, when found present in tissues or blood, function in part as activators of arginase, or what quantitative relationships exist between enzyme and metal concentrations *in vivo* remain to be determined. The exten-

sion of *in vitro* data to *in vivo* situations is always questionable.

Further information has been sought by the study of enzymatic activities in intact biologic systems, as exemplified by investigations on zinc-deficient animals by workers at the University of Wisconsin (E. Hove, C. A. Elvehjem, and E. B. Hart, *Am. J. Physiol.* **124,** *750 (1938)*; *J. Biol. Chem.* **136,** *425 (1940)*). Changes in enzymatic patterns, possibly characteristic of zinc deficiency, were sought and found. These alterations in enzyme activities were not reversible with the addition of zinc to the tissues *in vitro*. Similar results have recently been obtained in *Neurospora crassa* (A. Nason, N. O. Kaplan, and S. P. Colowick, *Ibid.* **188,** *397 (1951)*) where subsequent to zinc deficiency marked deviations in the pattern of some enzymes were observed.

The effects of metals in living systems seem subtle indeed. They may have eluded the investigator because his *mode* of observation has been inappropriate, not because his observational powers have been inadequately used. The exploitation of new avenues of approach to the problem will undoubtedly lead to the establishment of new criteria of appraisal which may facilitate our understanding of oligo-elements and their function.

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HEAT TREATMENT AND PROTEIN QUALITY

The browning or Maillard reaction, which occurs when proteins are heated in the presence of reducing sugars, has received the attention of many investigators in recent years (*Nutrition Reviews* **8,** *193 (1950)*). Since many foods are processed by heat treatment, the extent to which the Maillard reaction can influence the nutritive value of the product is of great importance.

Recently R. R. Baldwin, J. R. Lowry, and R. Thiessen (*Food Res.* **16,** *107 (1951)*) have determined the influence of heat treatment on casein in the presence of dextrose. Mixtures of equal parts of protein (casein N × 6.38) and dextrose, and one-half part water, were autoclaved for various periods and at various temperatures and pressures. The efficiency of utilization of the treated protein was determined in weanling rats by feeding a diet containing the protein at a 10 per cent level. The essential amino acid content of the treated proteins was determined microbiologically using *Leuconostoc mesenteroides* (P60) and *Streptococcus faecalis* as the organisms.

Processing for three minutes at 15 pounds pressure (121°C.) had reduced the protein efficiency (grams gain in rats per gram of protein consumed) by about 25 per cent. Similar processing for fifteen minutes resulted in such severe protein damage that the rats lost weight. Heating casein in the absence of the dextrose for extended periods (two hundred and eighty minutes at 15 pounds pressure) influenced its efficiency of utilization very little. Rats fed such casein gained 1.3 g. per gram of protein consumed, compared with 1.5 g. when unprocessed casein was fed.

The microbiologic assays indicated considerable destruction of some of the essential amino acids when casein was heated with dextrose for two hours at 15 pounds pressure. The extent of destruction was as follows (in per cent): arginine, 95; lysine, 80; tryptophan and methionine, 40 each; valine and histidine, 30 each; and isoleucine, leucine, threonine and phenylalanine, 15 to 20 each. Arginine and lysine destruction was 70 and 50 per cent, respectively, after only twenty minutes of such heat treatment.

The availability of the essential amino

acids in the heated casein-dextrose mixture was also determined for the rat. A product that had been heated for two hours at 15 pounds pressure was fed to rats at a 10 per cent dietary level. To each diet nine of the ten essential amino acids were added at levels as they appear in unprocessed casein at a 10 per cent dietary level. One of the essential amino acids was omitted from each diet. The growth responses obtained over a twenty-eight day period were compared with those obtained on a diet containing unheated casein.

These rat-growth tests indicated that the essential amino acids in the heated casein were less available than indicated by microbiologic assay. Threonine, for example, was almost completely unavailable for the rat, yet microbiologic assay indicated only 14 per cent destruction. Methionine and histidine were likewise unavailable for the rat whereas microbiologic assay indicated 50 to 60 per cent retention of activity. These results indicate that microbiologic assays cannot be used as reliable predictions of the value of heated protein for rats, a conclusion that was also drawn by L. Friedman and O. L. Kline on the basis of similar results (*J. Nutrition* **40,** *295 (1950)*). H. H. Mitchell and R. J. Block (*J. Biol. Chem.* **163,** *599 (1946)*) had also cited instances where the nutritive value of proteins was impaired by heat, although chemical methods indicated no alteration in amino acid content.

The rat assay method used by Baldwin and associates (*loc. cit.*) is, however, subject to some criticism. The addition of nine essential amino acids, as they appear in a 10 per cent level of dietary casein, on top of those already present in the heated casein in the diet might have resulted in amino acid imbalances that could influence the requirement of the one amino acid under test. It is well established, for example, that the minimum requirement for tryptophan in the rat is doubled when the dietary protein is increased from 10 per cent to 20 per cent. Several investigators have recently emphasized the importance of the level of dietary protein (total amino acids) in evaluating individual amino acid requirements (*Nutrition Reviews* **9,** *220 (1951)*).

Whether or not the decrease in nutritive value for the rat of the casein heated with dextrose was due to a decrease in digestibility was not determined in the studies by Baldwin and associates. Recent studies by I. J. Mader, L. J. Schroeder, and A. H. Smith (*J. Nutrition* **39,** *341 (1949)*) and by Schroeder, R. A. Stewart, and Smith (*Ibid.* **45,** *61 (1951)*) indicate that the *in vivo* digestibility for dogs is markedly decreased when lactalbumin is heated with lactose. Nitrogen balance studies indicated that all of the heat damage could be accounted for in the decreased digestibility and that the nitrogen absorbed from heated protein was as well utilized as that from unheated protein.

These results with heated lactalbumin-lactose obviously could not have been expected if one or more of the essential amino acids had been extensively destroyed, a situation which apparently prevailed under the conditions reported by Baldwin and associates with heated casein-dextrose. These apparently conflicting results may have as their basis the difference in behavior of lactalbumin and casein in the Maillard reaction. B. B. Cook, J. Fraenkel-Conrat, B. Singer, and A. F. Morgan (*J. Nutrition* **44,** *217 (1951)*) noted that maximum reaction between lactalbumin and lactose occurred after twenty-four hours at 52°C., whereas maximum reaction between casein and lactose required twenty-four hours of heating at 95°C.

These results serve to emphasize that there is still much to be learned regarding the influence of heat on protein-sugar mixtures. More extensive studies of the influence of the Maillard reaction on the digestibility of proteins in *raw* versus *processed* foods are definitely needed.

THE RELATION OF BODY FLUID COMPARTMENTS TO BODY FAT

In the care of seriously ill patients the physician is often faced with the problems of diminished or increased blood volumes. A reliable guide for estimating patients' "normal" blood volumes would be useful. A figure of 45 ml. per kilogram body weight has been often used, but the lack of close correlation between plasma volumes and body weight has long been recognized. A similar lack of correlation between total body water and body weight has been noted. This latter has been explained by the finding of an inverse correlation between total body water and body fat (N. Pace and E. N. Rathbun, *J. Biol. Chem.* **158,** *685 (1945)*; J. D. Hardy and D. L. Drabkin, *Fed. Proc.* **9,** *182 (1950)*). Hardy, P. K. Sen, and Drabkin (*Surg. Gynec. Obstet.* **93,** *103 (1951)*) have recently observed a similar situation in regard to plasma and possibly in regard to "extracellular fluid" (thiocyanate).

Observations were made in 8 healthy adult males of varying body stature. Total body water was measured with deuterium oxide, extracellular fluid with sodium thiocyanate and plasma with the dye T-1824. Body surface areas were estimated from DuBois' charts using measured heights and weights. "Ideal" weights were estimated by means of formulas in use at the Diabetic Clinic of the Hospital of the University of Pennsylvania.

It was found that plasma volume was more closely correlated with total body water than with surface area, height, or body weight, observed or "ideal." (The difficulties associated with estimated "ideal" weights have been reviewed, *Nutrition Reviews* **9,** *265 (1951)*; **10,** *14 (1952)*). Plasma volume was found to range from 2.75 to 5.65 per cent of body weight with a mean of 4.24 per cent and an average deviation of 22 per cent. When related to total body water, plasma volumes ranged from 6.2 to 7.8 per cent with a mean of 6.96 per cent and an average deviation of 7 per cent.

Regarding thiocyanate space, the data are less convincing, though it is stated by the authors that "from these and other observations not reported here it would appear that not only plasma volume but also thiocyanate space is more closely correlated with total body water than with body weight." This would be in keeping with the opinion of I. S. Edelman *et al.* ("Surgical Forum, American College of Surgeons," *p. 476, W. B. Saunders, Philadelphia (1951)*), who believe from their studies with deuterium that the plasma-interstitial fluid pool acts as a single chamber for water distribution. However, it was observed by Hardy *et al.* that the ratio of thiocyanate space to body water was greater in the obese subjects. Greater penetration of fat cells by thiocyanate, displacement of intracellular water to the extracellular phase by fat, and increased available space between the fat cells themselves were suggested as possible reasons for this observation.

It would appear that plasma volume, like total body water, is proportional to the lean body mass and inversely proportional to body fat of normal males. The situation regarding extracellular water awaits further study with other technics.

IMPROVING THE NUTRITIVE VALUE OF CORN

Corn is a major crop upon which much of our livestock industry depends, particularly that part dealing with hogs and poultry. The nutritive value of this crop is continually being studied, and methods of improving it are under investigation from several avenues of approach.

L. W. Aurand and R. C. Miller (*Fed. Proc.* **8,** *378 (1949)*) studied 10 corn hybrids and their single cross combinations, repli-

cated on 3 plots with the same fertilizer treatment. Single crosses ranged from 8 to 12.5 per cent crude protein and from 0.5 to 2.0 mg. carotene per pound, all expressed on the basis of 15.5 per cent moisture. These ranges are of considerable magnitude, and demonstrate possibilities for producing more nutritive corn by careful selection of hybrid seed stock and suitable crosses. The crosses showed a definite influence of inbred lines, both for protein and carotene, but there was no apparent correlation of protein with carotene contents. These results were considered very encouraging.

Niacin content has long been an important factor in corn studies (*Nutrition Reviews* **8,** *241* (*1950*)). F. D. Richey and R. F. Dawson (*Plant Physiol.* **26,** *475* (*1951*)) have recently conducted intensive experiments on the inheritance of niacin content in corn. Earlier studies had shown that niacin concentration in dent corn was determined by many genes of small individual effects. No data indicate that even large differences in kernel size modify importantly the niacin content per gram of dry matter. Niacin is not restricted to the endosperm, but since the endosperm constitutes 90 per cent of the corn kernel, any effect on niacin content of the endosperm would effectively determine the content of the whole kernel. Extensive new data prove that the niacin concentration of the endosperm is controlled exclusively by the direct action of genes during its formation and that environmental factors have a negligible influence. Also, the evidence of a 2:1 ratio for the effect of seed and pollen parents appears conclusive. Extremes for individual seed ears of 70 and 7 parts per million (p.p.m.) of niacin have been found. The niacin studied here is principally free nicotinic acid rather than a coenzyme or nicotinamide. It appears that higher-niacin strains of corn may be those having a balance of plus niacin genes to promote synthesis, or a balance of minus niacin genes to retard degradation, or both.

Selection for higher niacin among sugary segregates was successful in initiating strains of sweet corn with concentrations above those in the inbreds used at the start of these experiments, one selected ear having 92 p.p.m. of niacin. Studies with the starchy and sweet genes suggest that a higher-niacin sweet corn should have a lower starch content and consequently be sweeter and more tender. Niacin assay may thus be a useful tool in breeding sweet corn for improved quality as well as nutritive value. By use of embryo culture methods, assays may be made on endosperms of individual kernels and the corresponding embryos used to produce plants of specific phenotypes.

E. R. Leng, J. J. Curtis, and M. C. Shekleton (*Science* **111,** *665* (*1950*)) also report on the niacin content of sugary, waxy, and starchy corns in the F_2 segregating kernels. Sugary kernels averaged 23 per cent higher than waxy kernels, which were in turn 18 per cent higher than starchy kernels. These workers could not explain completely the apparent relationship between endosperm type and niacin content by assumptions of genetic linkage. They consider it more likely that there is a direct influence of endosperm type on niacin content of the kernel. Perhaps vitamin-carbohydrate relationships in the developing kernel are involved.

Sugary corn has also been found to be higher in total indoleacetic acid. Since niacin-indoleacetic acid interactions have been reported in plant growth, as well as tryptophan-niacin relationships in corn, H. J. Teas and A. C. Newton (*Plant Physiol.* **26,** *494* (*1951*)) were led to analyze 7 endosperm mutants and 8 inbred lines for niacin, indoleacetic acid and tryptophan. Two of the mutants contained exceptionally large quantities of niacin, 116 and 120 p.p.m., compared to the average value of 25 for the 8 inbred lines. Poor germination and susceptibility to mold damage under field conditions operate against the use of these particular mutants in a practical breeding program, but more suitable endosperm mutants may be found.

The inheritance of oil content in corn grain has been a difficult subject. This is important in fattening livestock and for the oil extraction business. Early work indicated that at least 20 genes condition oil percentage. A recent investigation by P. A. Miller (*Agronomy J.* **43,** *229 (1951)*) utilizes the technic of associating genes conditioning oil content with points of breakage of chromosomal interchange. Two inbred lines, representing extreme differences in oil content, were tested. By suitable crossing experiments and chromosome interchanges, at least one region of each of 9 different chromosomes was explored. In the low-oil backcross progenies there was little evidence of association between genes for low-oil content and points of chromosomal interchange involved. Data from the high-oil backcross progeny could be explained in two ways: (1) oil percentage is conditioned by several specific genes, or (2) it is conditioned by a large number of genes with small and approximately equal effects and distributed at random over the 10 pairs of corn chromosomes. The latter theory is favored by the over-all evidence available.

DIET AND POISONS

The influence of diet upon the relative toxicity of poisons has not been studied extensively. Important problems for future study are whether or not poisons can influence nutritional requirements and whether or not specific nutrients are involved in establishing resistance to poisons.

A review of the relation of nutrition to susceptibility to toxic substances (M. E. Shils, *Am. J. Pub. Health* **41,** *417 (1951)*) has emphasized the importance of these problems. H. H. Shrenk (*Ind. Eng. Chem.* **43,** (*Suppl. to Aug. issue*) *89A (1951)*) has pointed out the importance of well-balanced, adequate diets for workers who are exposed to industrial poisons. These reviews, however, make it clear that there is a striking lack of sound research data upon which to base the diet recommendations for workers exposed to occupational poisons. Studies conducted by industrial groups on humans have often yielded only confused results because of a lack of adequate controls or because of a lack of specific information concerning the nutritional state of the subjects under study. Up to the present, animal experiments have supplied the most promising information.

Poisoning from TNT (2,4,6-trinitrotoluene) has received considerable attention. This interest was stimulated by the poisoning of industrial workers by this compound during World War I. Recently M. E. Shils and L. J. Goldwater (*J. Nutrition* **41,** *293 (1950)*) have reinvestigated the effect of diet on the susceptibility of rats to poisoning by TNT. It has been assumed generally that well-balanced meals which contain adequate protein and are moderately low in fat should be recommended for workers exposed to this poison (*Nutrition Reviews* **8,** *127 (1950)*). H. P. Himsworth and L. E. Glynn (*Clin. Sci.* **4,** *421 (1942)*) demonstrated that high-fat diets had a markedly deleterious effect in rats consuming TNT. These high-fat diets were also low in protein.

In the recent study by Shils and Goldwater comparisons were made on the growth, appearance, blood picture, and pigment excretion in TNT-treated and control rats receiving 4 diets of varying protein, fat, and carbohydrate content. The basic diet ingredients were casein, corn oil, cerelose and salts. These ingredients were varied so that each of 2 diets (low-protein) contained 12.5 per cent of the total calories in the form of protein and each of 2 diets (moderate-protein) contained 25 per cent of the total calories as protein. One of the low-protein and one of the moderate-protein

diets contained corn oil to supply 60 per cent of the calories whereas the other 2 contained corn oil to supply 6 per cent of the total calories. The remainder of the calories in all diets was supplied by cerelose.

Vitamins were mixed into the diets in the following amounts per 400 calories of diet: thiamine, riboflavin and pyridoxine, 2 mg. each; calcium pantothenate and niacin, 5 mg. each; folacin, 0.2 mg.; biotin, 0.002 mg.; inositol and para-aminobenzoic acid, 2 mg. each; percomorph oil, 3 drops; and menadione and alpha-tocopherol, 1 mg. each.

The TNT was mixed into the diet at levels so that the rats each consumed between 21 and 24 mg. daily in the first series of studies. In the second series the TNT was increased in the diet by one-third after thirty-one days, so that average daily consumption over the first forty-five days varied between 24 and 28 mg. The studies were conducted over a ninety-day period. Six to 10 rats, approximately 70 g. in initial body weight, were used in each series of studies.

The diets containing 25 per cent of the calories in the form of protein afforded no more protection against TNT poisoning than did the diets containing 12.5 per cent of the calories in the form of protein. This conclusion was supported by the body weight data as well as by the blood hemoglobin data. TNT resulted in growth depression on all 4 diets and in all cases the growth obtained was about 40 to 50 per cent of that obtained on the same diets without TNT. This would imply that protein does not have any special detoxification effect against this poison.

The body weight data indicated that the toxicity of TNT was only slightly enhanced by the high-fat diets. In the absence of TNT, rats receiving the high-fat diets gained about 40 g. more than did those on the low-fat diets, whereas in the presence of TNT this growth-stimulating effect of fat was eliminated. In any event, the dietary fat effect on TNT toxicity could not be considered as being very great since growth was equal between the high-fat and low-fat diets containing TNT. The hemoglobin data failed to reveal any harmful effects from the high-fat diets. Hemoglobin values ranged between 10.1 and 11.4 g. per cent in the TNT-treated rats regardless of diets, and between 12.5 and 14.2 g. per cent in the controls.

These results thus are not in agreement with Himsworth and Glynn (*loc. cit.*), who reported a striking damaging effect of high fat diets in TNT poisoning. However, the diet employed by Himsworth and Glynn contained yeast and wheat and was thus considerably different from the diets employed by Shils and Goldwater. That this may have a bearing on the different results is emphasized by the recent report of R. H. Wilson and F. DeEds (*Arch. Indust. Hyg. Occup. Med.* **1,** *73 (1950)*). These workers found that diet A, as compared with diet B, greatly increased the toxicity of cadmium, moderately increased the toxicity of acetaminofluorene, failed to influence the toxicity of thiourea, and decreased the toxicity of xylose for rats. Analysis of the diets on a dry weight basis indicated the following composition in per cent for diets A and B respectively: protein, 16.2 and 21.0; crude fat, 7.2 and 4.1; crude fiber, 3.0 and 6.0; ash, 3.0 and 9.0; and nitrogen-free extract, 60.6 and 46.0. The diets were both made up of natural products and both presumably were adequate for the rat. The diets did not vary greatly in protein, carbohydrate and fat, yet the toxicities of the agents studied varied greatly depending upon which of the two diets was employed.

The results of Wilson and DeEds serve to emphasize that much is still unknown concerning the relation of specific nutrients to toxic agents.

FLUORIDE AND CARIES IN ADULTS

In discussions of the cariostatic effect of fluorine, emphasis has been placed upon the protection afforded to children by this element. Indeed, there is no general agreement that fluorine exerts an anticariogenic influence in adults. Further information regarding this question seems desired, and a recent study (A. L. Russell and E. Elvove, *Pub. Health Reports* **66,** *1389 (1951)*) of objective sample lists of adults who are native to Colorado Springs, and to Boulder, has been carried out. Both cities are similar from the geographic, climatologic and sociologic point of view; the water supplies in the two communities, however, differ greatly, for the water in Colorado Springs shows a mean annual content of fluorine of 2.55 parts per million (p.p.m.), whereas the water supplied to Boulder contains 0.1 p.p.m. or less of fluorine.

Considerable effort was put forth to secure data on adult natives of the two cities. The age limit of 20 to 44 years was set and the examination lists were checked for validity through school census records, birth records, marriage records, and city directories. The distribution of national background, occupational level, college experience, and financial standing was similar in the two groups. Dental examinations in both cities were made within a period of five months in 1950 in a central clinic using mirror and explorer; the outline of each lesion was recorded, information with respect to missing teeth and restorations was secured, and the condition of the gingival tissues was noted. No case of dental fluorosis was observed in the Boulder group, whereas 322 of the total 385 members of the Colorado Springs group showed very mild to severe fluorosis. The decayed, missing or filled (DMF) rates in both groups showed an increase with age, with the magnitude of increase somewhat greater in the Boulder group; however, the mean DMF rate for all ages was 17.2 in the Boulder (fluorine-free water) group and 7.5 in the Colorado Springs (fluorine-containing water) group. No person in the Boulder group was caries-free, whereas in Colorado Springs 26.4 per cent of the subjects in the 20 to 24 years age group, decreasing to 1.8 per cent in the 40 to 44 years age group, were caries-free.

In each age group there were more filled surfaces per filled tooth in Boulder than in Colorado Springs, and the rate of loss of teeth due to caries was more than three times greater in Boulder. The difference in DMF rates which are in favor of the Colorado Springs group are not altered by adjustment for sex, education or economic status of the subjects. The two groups appear to be comparable in every respect save for the continued use of fluoride-containing water in Colorado Springs and the use of fluoride-free water in Boulder. The data support the conclusion that the cariostatic effect of fluoride is exerted in adults as well as in children who are native to areas whose potable water contains this ion.

XANTHINE OXIDASE AND DIET

The liver has long been considered the locus of oxidative changes with respect to the purines, resulting, in man, in the appearance of uric acid as the end product of this metabolism. One of the enzymes taking part in this chain of chemical events is xanthine oxidase. It has been shown (W. W. Westerfeld and D. A. Richert, *Proc. Soc. Exp. Biol. Med.* **71,** *181 (1949)*) that the small intestine of the rat is relatively rich in xanthine oxidase, and that the content of both this tissue and the liver could be altered by dietary adjustment.

The details of one study in this direction have recently been published (W. W. Westerfeld and D. A. Richert, *J. Biol.*

Chem. **192,** *35* (*1951*)). Comparisons of the concentrations of xanthine oxidase in the upper small intestinal tissue and in the liver of young and adult rats were made using oxygen uptake as the criterion after the tissue homogenate was allowed to act upon hypoxanthine as the substrate. It was pointed out that this purine was used instead of xanthine itself because of the difficulty in securing satisfactory commercial samples of the latter. Purified diets were used with the concentrations of casein and of water-insoluble fraction of liver as the variable factors. It had previously been shown that this liver fraction contained an unidentified factor promoting the deposition of xanthine oxidase in liver and intestine of experimental animals (Westerfeld and Richert, *Ibid.* **184,** *163* (*1950*)).

It was observed at the outset that in young rats the distal portion of the small intestine had only 70 to 80 per cent of the xanthine oxidase activity of the proximal two-fifths. In the feeding experiments it was found that with respect to hepatic xanthine oxidase the level of dietary protein is of major importance, but that the unidentified factor in liver plays a part if the protein level of the ration exceeds the limiting value of 18 per cent. Intestinal xanthine oxidase, on the other hand, is responsive to both the protein content of the diet and the unidentified liver factor. It is pointed out that, for this reason, the variation of the level of the enzyme in the intestine would be superior as a device for demonstrating or assaying the liver residue factor in the diet. In adult rats, the only rations producing a drop in hepatic xanthine oxidase were poor in protein, the liver residue factor apparently having little if any influence. However, the enzyme concentration in the adult intestine responded to changes in levels of both dietary protein and the unidentified liver factor. Fasting results in a more or less parallel loss of xanthine oxidase in liver and intestine so that at the end of nine days without food the concentration of the enzyme in each tissue was reduced by one-half.

The foregoing observations have permitted the development of a method of assay for the unidentified dietary factor which promotes the increase in concentration of tissue xanthine oxidase (D. A. Richert and W. W. Westerfeld, *J. Biol. Chem.* **192,** *49* (*1951*)). It was found that when weanling rats were given a diet containing 21 per cent casein to which was added a known source of the unidentified factor (liver residue) in amounts from 0 to 15 per cent of the diet, the level of xanthine oxidase in the intestine varied progressively from 6 to 33, expressed as cubic millimeters of oxygen per twenty minutes per unit weight of homogenate of tissue (283 mg.). A considerable number of food materials and other compounds of biochemical interest were assayed for the xanthine oxidase-stimulating dietary factor, using the foregoing denomination and liver as a basis of comparison. The following materials have proved to be good sources of the factor: hog kidney, liver, spleen, aureomycin mash, buttermilk, cerophyl and soybean flour. Moderately good sources were milk and duodenum.

NUTRITIVE VALUE OF SOW'S MILK

The milk of different species of animals varies widely in composition (*Nutrition Reviews* **3,** *118* (*1948*)). The milk of sows has a high caloric value. Normally the newborn pig doubles its body weight in seven to ten days; this growth rate, in relation to size, is very high compared with that of other mammals. British workers have recently presented evidence to indicate that sow's milk may possess a growth factor or factors, or special growth-promoting properties other than that related to high caloric value (R. Braude, K. M. Henry, S. K. Kon, and K. G. Mitchell, *J. Dairy Res.* **18,** *283* (*1951*)).

In the first experiment 12 pairs of 3-week-old litter-mate male rats were used. One rat from each pair was fed cow's milk and the other sow's milk, which had been stored in the frozen state until needed.

The percentage composition of the sow's milk and cow's milk as collected was as follows: (values in parentheses are those for the cow's milk used) total solids, 19.27 (14.74); fat, 7.60 (5.88); solids-not-fat, 11.67 (8.86); protein, 5.62 (3.92); lactose, 5.12 (4.25); and ash, 0.93 (0.69). The sow's milk was diluted with water so that it contained approximately the same total solids as cow's milk and nearly the same caloric value. Both milks were supplemented with adequate quantities of minerals. The paired feeding technic was used so that the food intake between pairs was equal.

The rats receiving the sow's milk gained an average of 100 g. in body weight in four weeks as compared with 88.7 g. for those receiving cow's milk. Moreover, the rats fed sow's milk required 13.18 calories per gram of weight gain, as contrasted with a requirement of 14.77 calories for the rats fed cow's milk.

In a second experiment whole undiluted sow's milk was compared with evaporated cow's milk. The latter was diluted with water, and the caloric values per quart were 1189.1 and 1147.3 for cow's and sow's milk respectively. Although there was no difference in the total weight gain, the rats receiving the sow's milk again utilized the energy of the diet more efficiently. These rats consumed 13.99 calories per gram of body weight gain compared with 14.61 for those receiving cow's milk.

In a third experiment sow's milk and cow's milk were compared, with the former diluted again to equal cow's milk in caloric value. In addition, 2 more groups of rats were fed these same milk diets supplemented with the following vitamins: thiamine, riboflavin, pyridoxine, pantothenic acid, folacin, biotin, para-aminobenzoic acid, niacin, inositol, choline, vitamin K and vitamin B_{12}. Rats receiving sow's milk either with or without the vitamin supplement grew at a greater rate and utilized their feed more efficiently than the rats receiving cow's milk. The vitamin supplement to either milk was without significant effect on growth.

The biologic value and the digestibility of the proteins of "freeze-dried" samples of cow's and sow's milk were next determined. Rats were used as experimental animals. The biologic values were 85.1 and 90.3 per cent for sow's and cow's milk protein respectively. The digestibilities were 93.2 and 95.2 per cent for sow's and cow's milk protein respectively. The superior growth obtained in young rats on sow's milk could therefore not be attributed to superior protein digestibility or biologic value. Neither could the superiority of the sow's milk be attributed to any of the vitamins used in the study. These workers thus concluded that sow's milk contained some special properties or some unidentified nutritional factor or factors which enabled rats to grow rapidly and utilize energy efficiently.

It has been reported that sow's milk contains about six times as much total tocopherols as cow's milk. Sow's milk contains 18 mg. per 100 g. compared with 3 mg. per 100 g. for cow's milk (E. L. Hove and P. L. Harris, *J. Am. Oil Chem. Soc.* **28,** *405 (1951)*). Tocopherols were not included in the vitamin mixture tested by the British workers, and therefore whether or not the addition of tocopherols to cow's milk would improve it to the point where it would equal sow's milk is not known.

MECHANISM OF FLUORIDE ACTION

Recent preliminary reports from the Newburgh-Kingston study on the artificial fluoridation of communal water supplies strongly suggest that this procedure has a

similar effect on caries incidence to that seen in regions of natural fluoride-bearing waters (*Nutrition Reviews* **9,** *21 (1951)*). However, the mechanism by which fluoride incorporation into the tooth structure operates to reduce the susceptibility to tooth decay is unknown.

In one attempt to explore the mechanism of fluoride action, H. Berggren and H. Hedström (*J. Dent. Res.* **30,** *169 (1951)*) studied the effects of topically applied fluorides on the permeability of enamel in anesthetized dogs. Canine teeth were treated with either a 2 or 4 per cent sodium fluoride solution for 1 to 6 applications for a total interval of thirty to ninety minutes to simulate the circumstances in topical fluoride application to human teeth. After the completion of the topical treatment, the permeability of the enamel was tested by exposing the treated teeth and those of suitable controls to a tetanus toxin-fructose mixture where the toxin titer was 1:100,000 and the fructose concentration 20 per cent. This solution was maintained around the exterior of the crown for approximately forty hours. Then the teeth were extracted and a pulp suspension was prepared from each tooth. The test of the permeability of the enamel was based on the inoculation of nonimmunized mice with these pulp suspensions. The percentage of mice which died was used as the criterion to establish the approximate amount of tetanus toxin which had passed through the enamel and dentin into the pulp. On the basis of this test, no difference in permeability was observed.

In another type of experiment designed with similar intent, P. H. Suess and L. S. Fosdick (*J. Dent. Res.* **30,** *177 (1951)*) have compared the acid solubility of an untreated aliquot of finely powdered enamel with the acid solubility of another aliquot of powdered enamel which had been allowed to stand with a 2 per cent sodium fluoride solution for three hours at 37.5°C. The calcium-phosphorus ratios of the two samples were 2.05 and 2.01, respectively. Hence, it was concluded that most or all of the fluoride appeared to be combined as fluoroapatite and not as calcium fluoride. After the fluoride-treated enamel was dried, it was found to contain 1435 parts per million (p.p.m.) of fluoride whereas the enamel from which it was prepared contained 127 p.p.m. of fluoride. Aliquots of the two enamel samples were compared in tests of their acid solubility in buffers varying in pH from 4 to 7 at intervals of approximately 0.5 units. The amount of calcium dissolved during the period of exposure was used as the criterion for judging the degree of solubility. The curves for the solubility of the fluoridized enamel were similar in slope to the curves for untreated enamel; however, in all cases, the solubility curve for the fluoride-treated enamel at any given pH was much closer to the curve for the solubility of untreated enamel in the buffer at 0.5 pH units less acidic than to the curve for untreated enamel at the same pH. Hence, it was concluded that the fluoride-treated enamel was more resistant to acid solution than the untreated enamel.

The current acidogenic theory for the production of tooth decay was assumed by these investigators to be correct; they postulated that the mechanism by which fluoride incorporation into the tooth structure operated to reduce the susceptibility to tooth decay must be mediated through reductions in the acid solubility of the enamel. However, in this hypothesis, these workers have neglected to consider that the fluoride-treated enamel in their experiments contained in excess of 10 times the amount of fluoride reported by F. J. McClure and R. C. Likins (*J. Dent. Res.* **30,** *172 (1951)*) to occur in the enamel of teeth from individuals with continuous residence in Aurora, Illinois, where substantial reductions of tooth decay have been correlated with the presence of 1.0 to 1.2 p.p.m. of fluoride in the water supply. Whereas there was only a 35 per cent difference in fluoride content

between enamel from teeth formed in a nonfluoride area and enamel formed where the water contained approximately 1.0 p.p.m. of fluoride, the current investigation compared the acid solubility of two enamel samples which differed tenfold in fluoride content. The analogy between these two situations seems to be farfetched even without further consideration of the fact that one was *in vivo* and the other *in vitro*.

One of the real difficulties in studying the metabolism of fluorides has been the lack of a satisfactory procedure for the determination of fluorides in blood. F. A. Smith and D. E. Gardner (*J. Dent. Res.* **30,** *182 (1951)*) have described a revised method for the determination of fluorides in blood. For this analysis, 50 ml. of oxalated whole blood were introduced into a 200 ml. distilling flask; concentrated sulfuric acid was added and 140 ml. of distillate collected. Then the distillate was evaporated to dryness in a platinum crucible with calcium oxide as the fluoride fixative. The dry matter was ashed fifteen to sixteen hours and redistilled with perchloric acid as the acidifying agent until 125 ml. of distillate were collected. The salt-acid thorium method of H. A. Williams (*The Analyst* **71,** *175 (1946)*) was used for the determination of the amount of fluoride in the second distillate. Recoveries in 26 determinations of known amounts of fluoride, ranging from 5 to 80 micrograms, averaged 99 per cent and varied between 94 and 105 per cent with a standard deviation of 2.3 per cent.

At the time of the report, 84 blood fluoride analyses had been obtained on 35 normal dogs and 20 analyses on 13 normal rabbits. The dogs were fed once daily a diet containing 13 p.p.m. of fluorides and were bled two hours before feeding. The rabbits were maintained ad libitum on a diet which contained 3.4 p.p.m. of fluoride. The drinking water contained 0.06 p.p.m. of fluoride. The blood fluoride values varied from almost zero to approximately 70 micrograms per cent. The blood fluoride values for rabbits varied from about zero to 15.0 micrograms per cent. Eighty-one per cent of the values for dog blood and 80 per cent of the values for rabbit blood were below 10 micrograms per cent. Twelve per cent of the dog bloods and the remainder of the rabbit bloods were within the range of 10 to 20 micrograms per cent of fluoride. Only 4 of the dog bloods contained above 20 micrograms per cent of fluoride; all 4 of these samples were collected from dogs shortly after their admission to the colony.

One area in which blood determinations for fluorides would be very helpful is in the assessment of the toxicity and therapeutic value of a variety of fluoride-containing compounds but, as yet, this criterion has not been applied. J. W. Hein *et al.* (*J. Dent. Res.* **30,** *466 (1951)*) have studied the toxicity of a variety of fluoride-containing compounds and have determined the effect of the same compounds on the caries incidence in hamsters. Drinking water was prepared containing either: trisodium aluminum hexafluoride at a level which supplied 19.5 p.p.m. of fluorine; flural, a complex of aluminum, fluorine and sulphate, at a level supplying 16 p.p.m. of fluorine; potassium monophosphohexafluoride at two levels, 40 p.p.m. and 114 p.p.m. of fluorine; sodium monofluorophosphate at a level of 20 p.p.m. of fluorine; or sodium fluoride at three levels, 17.3, 12.3 and 8.9 p.p.m. of fluorine, respectively. These solutions were administered to littermate controls. Trisodium aluminum hexafluoride, flural, sodium monofluorophosphate and the two higher levels of sodium fluoride gave statistically significant, nearly maximal reductions in the caries scores. Neither level of potassium monophosphohexafluoride gave a statistically significant reduction in caries scores. Sodium fluoride at a level of 8.9 p.p.m. of fluorine gave an 80 per cent reduction in caries score as compared to the 94 per cent reduction produced by the 12.3 p.p.m. level of fluorine as sodium fluoride. Interesting differences in the intraperitoneal

acute toxicity of the various fluorides as compared to sodium fluoride on a fluoride content basis were: trisodium aluminum hexafluoride, 0.007 as toxic; fluoral, 0.6 to 0.5 as toxic; potassium monophosphohexafluoride, 0.1 as toxic; and sodium monofluorophosphate, 0.4 to 0.5 as toxic.

Further studies on the effect of complex fluorides on caries incidence in white rats were made by I. Zipkin and F. J. McClure (*J. Dent. Res.* **30,** *524 (1951)*). Rats on a cariogenic diet received equal quantities of 50 p.p.m. fluorine in their drinking water either as sodium fluoride, disodium monosilicohexafluoride, sodium monofluorophosphate, potassium monophosphohexafluoride and sodium trifluoroacetate. In addition, all but sodium trifluoroacetate were injected intraperitoneally into littermates at levels comparable to those received orally. A control group was given no fluoride by either route. At the end of ninety days all rats were killed, the teeth scored for caries, and the femurs and mandibles analyzed for fluorides. Incisors and molar crowns were separated into dentin and enamel and analyzed for fluorides.

Pronounced mottling of the incisors was noted in rats which received sodium fluoride, disodium monosilicohexafluoride and sodium monofluorophosphate orally or intraperitoneally, but mottling was not observed in the incisors of the rats which received the same amounts of fluorides as potassium monophosphohexafluoride or sodium trifluoroacetate by either route of administration. The three compounds which caused mottling caused a pronounced and similar reduction in caries incidence provided that they were administered orally. The compounds which did not cause mottling did not result in any reduction in caries incidence when given orally. None of the compounds reduced the incidence of caries when they were administered intraperitoneally. The fluorine in compounds which caused mottling of the incisor teeth had been absorbed in significant and comparable amounts by the bones and teeth. The complex compounds which did not cause mottling did not result in the deposition of fluorides in the skeletal and dental tissues.

The above studies clearly indicate that fluoride compounds are not identical in toxicity, in metabolism and in effect on the caries incidence even though used in amounts which are comparable insofar as their fluorine content is concerned. These studies should clearly point out the necessity to test each compound thoroughly in the laboratory before its use in an artificial fluoridation program is contemplated.

DIETARY TREATMENT OF LIVER CIRRHOSIS

One of the important and serious problems that face the clinician is an adequate means of demonstrating the structural changes in the liver that may result from the treatment of cirrhosis. Cirrhosis has been so often described as the severely scarred and knotted liver condition that is seen at autopsy, that the concept has developed that this disease is static and irreversible.

J. S. Levy, A. G. Kahn, and R. W. Talley (*South. Med. J.* **44,** *571 (1951)*) have recently emphasized that liver cirrhosis is a dynamic disease and that it is reversible under favorable treatment. A constantly changing picture of injury and recovery was noted in a series of 42 cases of advanced liver cirrhosis that have been under observation during a five-year period by these workers at the University of Arkansas School of Medicine. Levy and co-workers emphasize that the liver biopsy afforded a more reliable criterion of evaluating the course of cirrhosis than the usual laboratory liver function tests.

Three cases were reported in detail. Biopsies demonstrated the remarkable pow-

ers of regeneration and healing that can occur in the cirrhotic liver. For example, a liver with dense bands of fibrosis and with prominent fibroblast proliferation was observed to return to an essentially normal structure within a few months. Unfortunately, no definite information was given regarding the therapeutic procedure employed. The reader thus is unable to associate the liver changes with the nutritional status of the patient.

W. D. Davis (*South. Med. J.* **44,** *577 (1951)*) reported a critical study of the results of dietary treatment in 68 patients with liver cirrhosis. The biopsy technic was employed to determine the changes in the livers under treatment. This series of patients was unique in that a relatively large number (29 of the 68 patients) received treatment in the early stages of the disease.

The results demonstrated rather conclusively that early liver cirrhosis can be cured with a nutritious diet of 200 g. of protein, 500 g. of carbohydrate, 150 g. of fat (4150 calories), moderate vitamin supplement (one therapeutic capsule daily), and one-half ounce of brewers' yeast, three times daily. Additional methionine, 1 g. four times daily, or intravenous injections of liver extract did not appear to be of additional benefit in the cases of early cirrhosis.

Of the 39 cases with advanced liver cirrhosis, 12 exhibited pronounced improvement under treatment, whereas 12 more showed definite but less rapid improvement. Two additional subjects, who had ascites and marked fatty infiltration of the liver, made rapid recoveries under the favorable dietary regimen. These results thus demonstrate that many cases of advanced liver cirrhosis can be stabilized, and if such patients adhere to an adequate diet, they can be returned to useful endeavors.

The influence of the lipotropic factors, choline and methionine, on 20 hospitalized patients with advanced liver disease was investigated by J. O. Williams, D. Cayer, and W. E. Cornatzer (*South. Med. J.* **44,** *369 (1951)*). The rate of phospholipid turnover, an estimate of the ability of the liver to synthesize phospholipids, was measured by analyzing blood samples for radioactivity following the injection of radioactive phosphorus (P^{32}). Needle biopsies of the liver were also done routinely.

Ten-gram doses of choline or methionine produced a marked decrease in the amount of microscopically visible liver fat. In such cases the same treatment also produced an immediate rise in the rate of phospholipid synthesis as measured by phospholipid turnover in the blood. On the other hand, the lipotropic factors did not influence the liver structure in cases where scarring and necrosis, with little or no fatty infiltration, were the prominent features. Neither did the lipotropic factors produce any rise in phospholipid turnover in such cases. These workers concluded that the lipotropic factors are of definite value as adjuncts to other accepted therapeutic methods in the treatment of liver disease where fatty infiltration is present. Their results are a confirmation of the earlier observations by W. E. Cornatzer and D. Cayer (*Nutrition Reviews* **9,** *247 (1951)*).

The results of these three groups of investigators point to the importance of employing adequate technics, not only for the diagnosis of liver disease, but also for an honest evaluation of therapeutic results. The needle biopsy and the measurement of phospholipid turnover appear to be desirable, if not essential, clinical laboratory procedures for following the course of liver cirrhosis under treatment. It is no doubt because of a lack of critical criteria that many clinicians have doubted the importance of nutrition in the treatment of advanced cirrhosis.

A NEWER PLAN OF WEIGHT CONTROL

A recent account of an effort to assist obese individuals to lose weight describes the use of psychotherapy on a group basis (A. L. Chapman, *Pub. Health Reports* **66,** *725 (1951)*). In suggesting the promise of favorable results in attacking the problem of treatment of obese persons on a psychologic basis, it is emphasized that obesity is, to a very large extent, the result of overeating with diminished activity, and that the underlying cause of overeating is psychologic rather than physiologic. Only to a limited extent can obesity be validly related to endocrine dysfunction. It has been pointed out (S. Bayles, *Am. J. Med. Sci.* **219,** *104 (1950)*) that the simple gratification of a need for pleasure often revolves about food. To those who once were hungry, ample food represents a change of fortune; elaborate meals often are the means to indicate social and financial security; persistent overeating is frequently a habit born from the use of food to relieve nervous and emotional tension. Obviously, the obese person consumes more food than his body requires; it seems logical, therefore, to seek the genesis of obesity in nonnutritional, nonmetabolic or psychologic causes.

It is estimated that there are 30,000,000 people in this country who are 10 per cent overweight and 15,000,000 who are 20 per cent overweight. Inasmuch as diet therapy and medication have failed to control obesity on a long-term basis, recourse was had to a psychologic motivation. Groups were formed of obese persons who had been unsuccessful in losing weight by their own efforts. The only requirements for joining a group were an earnest desire to lose weight, and the approval of the applicant's physician. Each group was led by a person with experience in group leadership who could stimulate intelligent discussions leading to the conviction, on the part of the group members, that despite earlier failures, reduction of body weight was possible in their case. After the set period of sixteen weeks many of the members have continued to meet informally, thus enjoying mutual support in the business of weight control.

Although the group approach to weight control is in the exploratory stage, the problem of obesity in the nation's population is a pressing one. Among the policy holders of a large insurance company more than 7 per cent were 20 per cent or more overweight, and about one and one-half per cent were 40 per cent overweight. These data on excess body weight appear to be a steady statistic of the biometry of the modern American adult. The prevalence of body weights 10 per cent in excess of average weight for sex and height tends to increase directly with age. Repeatedly, studies have shown that the mortality rates are higher among obese individuals than in those of ideal body weights. Furthermore, obesity is associated with an increased prevalence of and death from the degenerative diseases. Life insurance statistics show conclusively that diabetes and obesity are closely tied together. The harmful influence of obesity in arthritis and in cardiovascular disease is a matter of record.

It appears, from the record, that neither the present lot nor the prognosis of the obese is a happy one. Further accounts of the experience with the group psychotherapeutic approach to reduction of body weight in the obese will be of great interest to those attempting to correct this "most frequent physical abnormality" found in our population.

VITAMIN B_{12} THERAPY OF HEMORRHAGIC SHOCK

Shock due to loss of blood (hemorrhagic shock) if treated early by whole blood transfusion (and prevention of further blood loss) is nearly always reversible. Treated late, hemorrhagic shock is often irreversible—blood pressure rises only temporarily despite continued transfusions and death from peripheral collapse ensues.

It has been suggested that dietary factors may influence the course of shock. W. M. Govier and his associates (*J. Am. Med. Assn.* **126,** *749 (1944)*; *J. Pharmacol. Exp. Therap.* **72,** *317 (1941)*; **79,** *240 (1943)*; *Nutrition Reviews* **3,** *89 (1945)*; **9,** *204 (1951)*) observed that the resistance of normal dogs to hemorrhagic shock was increased when thiamine was given in large doses before or very soon after bleeding; dogs deficient in thiamine were especially susceptible to hemorrhagic shock. A possible explanation for these findings was suggested by their observation that a dephosphorylation of cocarboxylase occurs in shock and that thiamine therapy results in resynthesis of cocarboxylase. L. I. Katzin and S. L. Warren (*J. Clin. Invest.* **24,** *152 (1945)*), on the other hand, observed no accentuation of the mortality from tourniquet shock by thiamine deficiency in rats. The difficulty of comparing these results with those of Govier has been pointed out in an earlier review (*Nutrition Reviews* **3,** *316 (1945)*), *i. e.*, different experimental animals studied, different types of shock, and different criteria for effectiveness.

Decrease in adrenal vitamin C content, plasma ascorbic acid concentration, urinary excretion and tissue "saturation" (as judged by load tests) have been reported in hemorrhagic, traumatic and burn shock. A decrease in the urinary excretion and "saturation" of thiamine, riboflavin, and nicotinamide have also been reported under similar circumstances (*Nutrition Reviews* **5,** *141 (1947)*). The therapeutic implications of these findings have not been established.

Studies by J. Fine, A. M. Seligman, and H. A. Frank (*Ann. Surg.* **126,** *1002 (1947)*) have indicated that impairment of liver function may be one of the important factors leading to the irreversibility of hemorrhagic shock. It has recently been demonstrated by D. Koch-Weser and associates (*Proc. Soc. Exp. Biol. Med.* **71,** *688 (1949)*; *J. Lab. Clin. Med.* **36,** *694 (1950)*; *Nutrition Reviews* **9,** *133 (1951)*) that vitamin B_{12} when administered prophylactically in relatively large dosage will lessen liver injury induced by carbon tetrachloride. The vitamin was not effective when given only after liver injury had occurred. Considering that vitamin B_{12} might be concerned with maintenance of liver function in shock, J. M. Howard and M. E. DeBakey (*Surgery* **30,** *161 (1951)*) attempted to test the efficacy of vitamin B_{12} in the prophylaxis and treatment of irreversible hemorrhagic shock.

Shock was produced in dogs under pentothal anesthesia by bleeding them to a blood pressure of 30 mm. Hg. The rate of bleeding was relatively uniform and determined by the unrestricted flow of femoral arterial blood through a large standard size cannula. By subsequent small bleedings, and later by small transfusions, the blood pressure was maintained at 30 mm. Hg. until the time when twice within a period of twenty minutes a 25 cc. transfusion failed to maintain the pressure at or above 30 mm. Hg. Immediately thereafter all of the remaining collected blood was reinfused and the animal was given 300,000 units of penicillin intramuscularly. Heparin, 2.5 mg. per 100 cc. of blood, was used as the anticoagulant.

Each of 28 dogs was given intravenously 900 micrograms of vitamin B_{12}, dissolved in 15 cc. saline solution and given at the rate of 75 micrograms per ten-minute period either during the shock procedure (21 dogs)

or immediately following the retransfusion (7 dogs).

The shock pattern and forty-eight-hour mortality (82 per cent) of the two groups of vitamin B_{12}-treated animals were not significantly different from the results obtained in control dogs (forty-eight-hour mortality rate, 86 per cent) or in fifty dogs given 50 to 200 mg. of cortisone (forty-eight-hour mortality rate, 82 per cent). The cortisone was administered usually as a single injection at variable times during the experiment, from forty-eight hours prior to the shock procedure to immediately after retransfusion. The authors conclude that neither vitamin B_{12} nor cortisone has any significant effect upon the course of hemorrhagic shock under the conditions of their study. It should be pointed out, however, that the control dogs were composed of two series of test animals, 15 dogs studied at the time of the vitamin B_{12} and cortisone experiments, and 20 dogs studied previously (exact time not stated). The forty-eight-hour mortality of the latter group was 75 per cent, of the former 100 per cent. An average mortality of 86 per cent was used for comparison with the 82 per cent obtained in the vitamin B_{12}- and cortisone-treated dogs. It is conjectural whether the averaging of the two control series is sound. The dangers of comparing results of shock experiments carried out at one time with those carried out subsequently, or previously, have been emphasized by O. Glasser and I. H. Page (*Am. J. Physiol.* **154,** *297 (1948)*). It should also be noted that very large amounts (900 micrograms) of vitamin B_{12} were given and the authors state: "It was found that if the drug was given rapidly a fall in blood pressure occurred, so that in order to avoid influencing the criteria for terminating the experiment 7 animals were given the drug only at the completion of the shock procedure." It may be inferred that some hypotension specifically related to the administration of the vitamin B_{12} preparation occurred. What effect this may have had on the final outcome cannot be stated. It should be noted that using a somewhat similar type of hemorrhagic shock, H. A. Frank, A. M. Seligman, and J. Fine (*J. Clin. Invest.* **24,** *435 (1945)*; **25,** *22 (1946)*; "Surgical Forum of the American College of Surgeons," *p. 522, W. B. Saunders, Philadelphia (1951)*) found no agent among the many which they studied (including various vasopressor drugs, ACTH, and cortisone) therapeutically effective when given at the "irreversible" stage other than viviperfusion of the liver of the shocked dog.

EVALUATION OF PROTEIN QUALITY

From a nutritional point of view the quality of a protein depends upon the presence and availability of the amino acids, particularly those amino acids that are synthesized to a limited extent or not at all by most animals. In the past the nutritional quality of a protein has usually been evaluated by one of two biologic methods, the nitrogen balance method (per cent of absorbed nitrogen that is retained in the body); or the protein efficiency method (unit of body weight gain per unit of protein consumed). In recent years analytic procedures have been devised that permit fairly accurate determinations of the amino acids present in proteins or foods. Such methods, however, reveal nothing concerning the availability of the amino acids present. It is thus implied that any true assessment of the nutritional value of proteins must rely upon biologic evaluations.

Recently a rapid biologic method for assessing protein quality has been developed (M. E. Anderson and H. H. Williams, *J. Nutrition* **44,** *335 (1951)*). *Tetrahymena geleii* W, a protozoan form

which requires the same ten amino acids that are essential for the rat, served as the experimental animal. The H strain of this organism had been used for the determination of protein quality by M. S. Dunn and L. B. Rockland (*Proc. Soc. Exp. Biol. Med.* **64,** *377 (1947)*; *Food Technol.* **3,** *289 (1949)*; *J. Biol. Chem.* **179,** *511 (1949)*). These workers measured the growth response of the organisms to various proteins by titration of the acid produced in the experimental cultures after a forty-one day incubation period.

The authors sought to develop a more rapid technic, based upon the quantitative enzymatic reduction by *Tetrahymena geleii* W of colorless water-soluble 2,3,5-triphenyltetrazolium chloride to the red, water-insoluble triphenylformazan. The latter compound can be extracted from the culture with acetone and its concentration determined by colorimetric methods.

The procedure consisted of the following: Duplicate levels of graded amounts of a stock culture of the organism were pipetted into 15 ml. centrifuge tubes and a one-per cent solution of triphenyltetrazolium chloride (pH 7.7) was added in the ratio of 1 ml. to 5 ml. of culture fluid. After four hours of incubation at 25°C., mercuric chloride was added to each tube to stop the enzymatic reaction. The tubes were then centrifuged, the supernatant liquid discarded, and the colored triphenylformazan was extracted from the residue with acetone. There was a direct linear relationship between the concentration of the colored compound produced and the amount of stock culture used (the number of organisms present).

In order to test the validity of the method the organism was grown in basal media containing all but one of the ten essential amino acids and graded levels of the missing amino acid. All ten of the essential amino acids were tested in this manner. In all cases there was good agreement between the concentration of the amino acid in question and the growth response of the organism.

The usefulness of the method in testing protein quality was also studied. Liver meal, casein, whole egg and soybean meal were incorporated into the basal medium from which the amino acids had been omitted. Homogenization of the protein preparations appeared to be necessary in order to obtain maximum growth response. The organisms were incubated, with the media containing known amounts of the protein homogenates, for a period of five days at 25°C.

The growth response of the organisms differed markedly for each of the proteins tested. For example, casein produced optimum growth when present in a concentration to supply approximately 5 mg. of nitrogen per 25 ml. of incubation solution, whereas maxima with whole egg and soybean meal were not reached at a nitrogen level of 10 mg. per 25 ml. of incubation solution.

The authors point out that further studies on the interrelationship of culture incubation time and nitrogen level will have to be made before the medium can be used to assess biologic values of unhydrolyzed proteins.

Since the methods presently available for determining the biologic value of proteins are time-consuming and tedious, it is hoped that the method proposed by Anderson and Williams can be refined in order to serve as a reliable means of assessing protein quality.

EFFECT OF AMINOPTERIN ON EPITHELIUM

Folacin has an effect on growth promotion in intact animals and on cell proliferation of tissues. Antagonists of the vitamin have been shown to inhibit growth of bacteria, tumors, and experimental animals (*Nutrition Reviews* **8,** *282 (1950)*; **9,** *24 (1951)*).

The biologic roles of folacin which account for its effect on cell proliferation apparently relate to functions of the vitamin in nucleic acid and protein synthesis (*Ibid.* **8,** *20, 260 (1950)*; **9,** *185 (1951)*).

R. Gubner (*Am. J. Med. Sci.* **221,** *169 (1951)*) has compared the effect of a folacin antagonist, 4-aminopteroylglutamic acid (aminopterin), on cell proliferation and tissue reactivity with that of cortisone and ACTH. In experiments in which arthritis was induced by injections of formaldehyde in the periarticular tissues of albino rats, it was found that administration of 10 micrograms of aminopterin daily by mouth suppressed the inflammatory changes observed in and about the joints. This effect was stated to be similar to that observed when cortisone was administered to other animals with formalin arthritis. That the protection afforded by aminopterin is not mediated through the adrenals was demonstrated when it was found that inhibition of inflammatory reactions in formalin arthritis occurred in adrenalectomized rats given this antagonist of folacin. Unfortunately, the details of these experiments are not recorded and the observations are described very briefly.

These findings were utilized as the basis for a clinical trial of aminopterin administered to patients with arthritis (R. Gubner, S. August, and V. Ginsberg, *Am. J. Med. Sci.* **221,** *176 (1951)*). Seven patients with rheumatoid arthritis and one with acute rheumatic fever were given 1 to 2 mg. of aminopterin daily for a total dosage of from 6 to 40 mg. In 7 of the 8 patients there occurred symptomatic relief and subsidence of acute manifestations of exudative reactions in and about involved joints. There also was a decrease in fever and in leukocytosis. However, the long-term course of the disease was not influenced by aminopterin, and exacerbation of arthritic symptoms and signs occurred several weeks after administration of the antagonist had been discontinued. It is of some interest that a second course of therapy was associated with remission of symptoms.

One of the patients included in the group of patients with arthritis also suffered from extensive and long-standing psoriasis. Coincident with treatment with aminopterin there was subsidence in joint symptoms and striking improvement in the skin lesions of psoriasis. This led the authors to a more extensive clinical trial of aminopterin in treatment of psoriasis and an evaluation of the effects of the antagonist on epithelial tissues. The results of these experiments are recorded in a recently published report (R. Gubner, *Arch. Dermat. Syph.* **64,** *688 (1951)*).

Thirteen patients with skin lesions of psoriasis which had been existent for long periods of time and covered extensive areas of the body were given 1.5 to 2.0 mg. aminopterin daily until a total of 14 to 28 mg. had been administered. In all patients so treated there were significant changes in the skin indicating healing of the psoriatic lesions. The first observed manifestation of healing was cessation of scaling. This occurred within five to ten days of the beginning of aminopterin therapy. The dried scales of involved skin then separated, leaving a clear base of skin with purplish discoloration which faded on pressure. There was gradual disappearance of discoloration leaving skin of fairly normal appearance. Itching, which was usually a distressing symptom before therapy, decreased in intensity or no longer occurred. The characteristic signs and symptoms of psoriasis recurred in each patient one to two months after cessation of treatment with aminopterin. Second and third remissions of the disease were induced promptly by resumption of therapy with the folacin antagonist. None of the patients was observed for periods longer than nine months.

Five patients with skin lesions of an in-

durative type, in contrast to the desquamative lesions of psoriasis, were similarly treated. The author claims significant therapeutic effects from aminopterin administered to these patients. Included were 2 patients with chronic eczematoid dermatitis, one with chronic discoid lupus erythematosus and 2 with scleroderma.

The clinical experiences indicated, also, that aminopterin had an adverse effect on wound healing. In one instance in which a biopsy was taken of a subcutaneous nodule, the skin wound did not heal until three weeks after discontinuance of treatment with aminopterin. In other patients with decubitus ulcers and pustular infections in which infection was effectively controlled, lesions failed to heal until aminopterin was withdrawn.

Two other manifestations of the effect of aminopterin on epithelial tissues were noted. These included the development of ulcers of the buccal mucosa and thinning of the hair during administration of the antagonist. Ulcerations in the mouth occurred in all patients after a total of 10 to 40 mg. aminopterin had been administered. These usually occurred at a time when there were manifestations of healing of the dermatologic disorder treated. They often were accompanied by complaints of soreness of the mouth and throat, abdominal cramping, and diarrhea. The author claims that the ulcers occur because of inhibition of cell proliferation of the epithelial linings of the mouth and gastrointestinal tract with resultant ready development of superficial ulceration and infection. Such ulcerative lesions and associated symptoms became the limiting factors in aminopterin therapy in these patients. The mouth lesions healed spontaneously within a few days of termination of dosage of the drug. The author states that aureomycin given in prophylaxis lessened these disturbing toxic manifestations.

Seven of the patients had thinning of the hair or incomplete alopecia occurring with administration of aminopterin. Male patients stated that they found it necessary to shave less often. These phenomena developed several weeks after the appearance of mucosal ulcerations and usually after the drug was discontinued. There was regrowth of hair within two to three months.

Aminopterin acts to prevent the conversion of folacin to citrovorum factor, the metabolically active derivative (H. P. Broquist, E. L. R. Stokstad, and T. H. Jukes, *J. Biol. Chem.* **185,** *399 (1950)*). In the clinical observations of Gubner (*loc. cit.*) it was possible to prevent or to control mucosal ulcerations and other manifestations of toxicity of aminopterin by the intramuscular injection of a solution containing citrovorum factor. Moreover, when citrovorum factor was given, the beneficial effects of aminopterin in the dermatologic disorder were inhibited. Although leukopenia has been reported to occur frequently as a toxic manifestation of aminopterin administration, it was not found in any of the patients in this study.

It should be pointed out that claims have been made in the medical literature for the beneficial effects of a variety of agents in the treatment of psoriasis. None of these has proven wholly effective, and any evaluation of therapeutic effect in a disease associated as this one is with spontaneously occurring remissions and exacerbations must be carefully appraised. There is no proposal in this report that aminopterin is a suitable agent for the treatment of psoriasis. The data presented do indicate rather clearly, however, that folacin has a significant role in maintenance of health and integrity of epithelial tissues, and that antagonism of this action produces grossly demonstrable changes in normal mucosa as well as in skin, which is the site of a variety of disorders.

ANIMAL VERSUS VEGETABLE FOODS IN NUTRITION

The comparative merit of animal and vegetable foods in nutrition was the subject of the British Nutrition Society, London, England, at the 1951 scientific meeting (R. C. Garry, *Brit. J. Nutrition* **5,** *243 (1951)*). The many facets and the controversial aspects of this interesting problem were set forth in several papers.

K. J. Carpenter (*Brit. J. Nutrition* **5,** *243 (1951)*), in discussing the relative nutritional values of animal and vegetable proteins, has stressed a very important aspect of nutritional problems in undeveloped areas of the world. He pointed out that vegetable proteins may have to be relied upon heavily for animal feeding in such undeveloped regions where fish meal, meat meal, and milk products are not normally available.

Conversion factors for vegetable and animal food for human consumption were discussed by K. L. Blaxter (*Brit. J. Nutrition* **5,** *250 (1951)*). Blaxter has attempted to compare the food value of an acre of land producing animal matter for human consumption with that of an acre producing vegetable matter for human consumption. Energy, amino acids, calcium, vitamin C, thiamine, and vitamin A were considered in the comparison. An acre of good land sown to wheat was estimated to supply the caloric needs of 2.4 people for a year, the lysine needs for only 1.8 people, little or no ascorbic acid or vitamin A, and only enough calcium to meet the needs of 0.3 people for a year. In contrast, milk produced from one acre of good land was estimated to supply the lysine needs of 4.1 people, the calcium needs of 3.6, the vitamin A needs of nearly 2.0 people and the caloric needs of 0.6 people for a year.

In other words, these observations support the view that the main vegetable crops eaten by human beings supply a superabundance of energy, but their total nutritive value for humans is limited by their shortage of vitamins and essential amino acids. The animal products usually supply a superabundance of vitamins and essential amino acids per unit of energy. It is thus concluded that, in dealing with the conversion of plants and animals into human food, it is not their comparative merits but rather their complementary merits that are important. That is to say, an agricultural system devoid of animals is not an efficient method of using land for the production of human food.

These problems are of particular interest in areas of the world where relatively large populations are concentrated into relatively small land areas, such as the British Isles. The answer sought is obviously to increase the efficiency of utilization of home-grown foods for human consumption. D. A. Boyd of the Rothamsted Experiment Station (*Brit. J. Nutrition* **5,** *255 (1951)*) has calculated that only 9 per cent of the energy value of foods supplied to livestock in England is returned in the form of animal products for human consumption. From experimental data on the efficiency of conversion of food by farm animals, Boyd concludes that this value can be increased by 20 to 25 per cent through improved animal feed production programs and livestock management practices.

The general problem of vegetable protein improvement was reviewed by H. Chick (*Brit. J. Nutrition* **5,** *261 (1951)*). Many examples of complementary effects of protein mixtures have been noted. An interesting one mentioned is that beef tea (a hot beef extract containing mainly gelatine) and wheat are excellent protein sources for the growing rat when fed together, but do not permit good growth when fed separately.

Less well known complementary action mentioned by Chick is the relation between the proteins contained in the outer layers (bran) and the inner portions (endosperm) of wheat grain. Rats fed these proteins separately grow less well than rats receiving a mixture of these proteins. Also reported by

Chick was the interesting observation that the nonprotein nitrogen in potatoes, which amounts to about one-half the total, has a marked stimulatory effect when fed with potato protein (tuberin). This same stimulatory effect of the nonprotein nitrogen of potatoes was also noted when it was fed as a supplement to a diet containing wheat gluten as the protein source.

In summarizing the present information with regard to vegetable protein supplementation, Chick considers mixtures of cereals with soybeans as most promising. The mutual advantage of such mixtures often produces a nutritive value comparable to that of milk proteins. The particular value of soybeans seems to lie in their high content of lysine, an amino acid that is usually limiting in cereal proteins.

A paper by L. Wills (*Brit. J. Nutrition* **5,** *265 (1951)*) is devoted to a report of the clinical findings in children fed on a predominantly vegetable diet immediately after weaning. The cases studied consisted of 36 infants of the South African Bantu race suffering from malignant malnutrition, also known as Kwashiorkor or fatty liver disease. This condition was considered by Wills to be the extreme expression of the ill effects of diets consisting mainly of kaffir corn and corn meal porridge. This condition has been described previously (*Nutrition Reviews* **8,** *55, 161 (1950)*). Wills noted that the forced feeding of skim milk curds with skim milk powder resulted in rapid recovery. Within a few days the livers decreased in size, the edema was alleviated, and the low serum protein increased rapidly toward normal. Since it has yet to be demonstrated that Kwashiorkor can be prevented with a diet of mixed vegetables adequate in calories, Wills expresses her belief that the causative factor in this disease is a deficiency of animal protein. Of interest in connection with such a view was the report by R. F. A. Dean (*Brit. J. Nutrition* **5,** *269 (1951)*), who commented that vitamin B_{12} is present in all food substances that have been reported to be of curative value in Kwashiorkor.

In attempting to summarize this comprehensive conference devoted to the general subject of plant versus animal foods for humans, one conclusion is important, and apparently a very logical one. A judicious use of combinations of various plant foods, plus the insurance of an adequate vitamin B_{12} intake, would no doubt relieve the sufferings of millions of undernourished children in many sections of the world. This conclusion obviously points toward a need for more studies to determine the mutual benefits that can be derived from simple variety in inexpensive diets of predominantly vegetable origin. Such diets are particularly in demand in those areas of the world where population density forces an economy in which vegetable foods, because of their more efficient production, would take precedence over animal foods.

ACTIVITY OF PEPSIN

It has been said that the beginning of wisdom in the field of nutritional biochemistry is a good knowledge of gastrointestinal physiology. Thus, the various aspects of the activity of digestive enzymes, of the digestive hormones, and of the part played by peristalsis, are some of the legitimate interests of the well-trained nutritional biochemist. Accordingly, attention is directed to a recent study of the pattern of hydrolytic cleavage brought about by the protease, pepsin (B. T. Currie and H. B. Bull, *J. Biol. Chem.* **193,** *29 (1951)*). The pertinent data were secured by adapting physical chemical methods employed in the study of macromolecular monolayers to the examination of

the products of the digestion of crystallized egg albumen by crystalline pepsin. An extrapolation of a plot relating the product of the film pressure and film area (per unit of weight of peptide) with the film pressure yields on calculation the molecular weight of the peptide which has been spread as a film on a layer of salt solution. With a mixture of peptides such as would result from an enzyme proteolysis, the determination of the average molecular weights of groups of peptides of similar molecular size can be made by compressing the film in regular time intervals, at each state of which the smaller peptides drop out of the film and a new measurement of film force is made. It is thus possible to secure information on the molecular weight distributions of the peptides resulting from the hydrolytic process.

The influence of various environmental factors upon the rate and extent of peptic proteolysis was re-examined. The influence of pH was unmistakable with respect to the number of peptide bonds of the substrate which were hydrolized. Whereas enzyme activity occurs from pH 1.7 to 2.85 in these experiments, the molecular weight in the peptides in the surface layer measured after film compression indicated that at pH above 2.25 the products of the hydrolysis possess distinctly lower molecular weights than do those formed at lower pH. It appears that pepsin acts as a proteinase and splits fewer peptide linkages per mole of substrate at low pH whereas at a higher pH it acts as a peptidase and yields fragments of smaller molecular weights. These interrelations of enzyme and substrate appear to be mutually exclusive.

The length of time of digestion by pepsin has little influence on the ultimate weight distribution of peptides. However, as the quantity of pepsin in the system increased there was a progressively increased degree of fragmentation of the substrate. With respect to temperature, there appeared to be a differentiation in the activity of pepsin; at temperatures below 30° C. the peptides accumulating in the surface were of relatively low molecular weights whereas at 35°, 40°, and 50° C. the products of the hydrolysis were of high molecular weights.

The hydrolysis of egg albumen by pepsin is much like that produced by hydrochloric acid as regards the weight distribution of the resulting peptides. The persistence of high molecular weight but noncoagulable peptides along with smaller fragments suggests that the first action of pepsin is an activation of the substrate—a sort of denaturation—with subsequent hydrolysis to large peptides if the pH is low and temperature high, and to smaller peptides if these environmental factors are reversed. It is of considerable interest that despite the fact that a crystalline protein enzyme was employed it appears to have at least two distinct, if related, functions.

LIVER FAT AND ANTIBIOTIC THERAPY

The antibacterial potency of the newer antibiotic agents in human infections appears to be well established, but the possible deleterious effects of these agents when used in large doses over long periods of time have not yet been elucidated. From the standpoint of nutrition and intermediary metabolism there is suggestive evidence that aureomycin and chloramphenicol may precipitate vitamin deficiency signs in the human (*Nutrition Reviews* **9**, *283* (*1951*)). Several recent reports now indicate that the administration of aureomycin and terramycin may produce fatty infiltration of the liver although chloramphenicol is less potent in this respect.

Preliminary observations on changes in liver morphology during therapy with sev-

eral antibiotic agents have been made by R. Yesner and P. Kunkel (*Yale J. Biol. Med.* **23,** *299 (1951)*). Four patients were studied by liver biopsy before and after oral aureomycin therapy for various infections. Although none had significant fat infiltration before therapy, 3 patients on whom biopsies were obtained immediately after the course of treatment showed fat vacuolization, while one patient whose biopsy was not done until sixteen days after cessation of therapy showed no change. Subsequent specimens in 2 of the patients with positive findings revealed decreasing infiltration five and forty-four days after aureomycin was stopped. Liver function tests done in 2 patients before and during treatment showed improvement despite the development of fat vacuoles. Similar biopsy studies on 3 patients receiving tibione, on 4 receiving combined tibione, streptomycin and para-aminosalicylic acid, and on one receiving chloramphenicol showed no evidence of morphologic change.

In reference to the results of tibione therapy it is worth noting the studies of A. Falk (*Tr. Ninth Streptomycin Conference April 18–21, 1950, Veterans Administration, Washington, D. C., p. 83*). Of 15 tuberculous patients receiving tibione, 8 showed increased bromsulfalein retention after sixty to ninety days of treatment. Liver biopsies performed on 4 patients revealed increased amounts of fat. As in the investigation of Yesner and Kunkel, however, no dietary intake data were reported.

In a more carefully controlled study V. M. Sborov and D. A. Sutherland (*Gastroenterology* **18,** *598 (1951)*) investigated the changes in liver biopsies, liver function tests and body weight in 16 patients receiving aureomycin and in 3 receiving terramycin. All subjects had previously had diagnoses of either chronic hepatitis or cirrhosis and were fed similar diets containing approximately 102 g. of protein and 3000 calories supplemented by two multivitamin capsules daily. Aureomycin or terramycin was administered in doses of 2 g. daily for periods of thirty to sixty days (aureomycin) or thirty-seven days (terramycin). Seven of the 16 patients receiving the first drug and 2 of the 3 who were given terramycin showed significantly increased liver fat. The duration of antibiotic treatment did not appear to influence the amount of fat in the liver, and correlation between increased abnormality of liver function tests and fatty infiltration was not demonstrable.

In a larger series of cases, in which liver biopsies were not done, the effect of aureomycin given intravenously alone or in combination with oral medication on gross hepatic function has been studied (M. H. Lepper *et al., Arch. Int. Med.* **88,** *271 (1951)*). These workers observed 103 patients of whom 89 received aureomycin intravenously alone in daily doses of 6 to 36 mg. per kilogram of body weight. The remainder received aureomycin well in excess of 40 mg. per kilogram from combined oral and intravenous dosage. Although no biopsies or serial liver function tests were carried out, no clinical evidence of liver dysfunction in the form of hepatic enlargement or jaundice was noted in the first group. Of the 14 patients receiving both oral and intravenous antibiotic, however, 7 showed hepatic enlargement while 4 of these demonstrated jaundice and one had hyperbilirubinemia. Examination of the liver at autopsy in 5 and by liver biopsy in one of these 7 patients revealed fat vacuolization in all, with some cases also demonstrating fragmentation of the liver cytoplasm. It should be pointed out, however, that all were suffering from severe infections of various types, and one patient who showed evidence at autopsy of liver damage was also found to have cirrhosis.

On the basis of this rather large series of studies on humans the authors suggest that intravenous aureomycin should be given in doses limited to one gram daily in adults also receiving oral therapy, and when given alone,

intravenous dosage should not exceed 2 g. For children, 40 mg. per kilogram per day is proposed as a maximum intravenous dose. Although the evidence of liver damage as a result of aureomycin is suggestive in this investigation, control data such as pretreatment biopsies or function tests are extremely limited, and no information concerning dietary intake is presented.

In an extension of their clinical studies, these workers have also conducted a series of experiments on mice and dogs (M. H. Lepper *et al.*, *Arch. Int. Med.* **88,** *284 (1951)*). In the initial study, 47 mice receiving aureomycin (80 to 100 mg. per kilogram) intraperitoneally for seven days showed significant fat vacuolization in the livers of 30, while none of the 48 saline-treated control group demonstrated this change. In a second series of experiments the drug was given buffered and unbuffered intraperitoneally, and subcutaneously in the buffered form. Suitable controls were used to eliminate the effect of the buffering agent and possible intraperitoneal irritation. Again, only those animals receiving aureomycin showed significant liver changes. Food intake was then controlled in two other sets of studies, one in which the mice were fed in groups in cages and another in which the animals were housed individually and pair-fed. Even with this control of food intakes aureomycin was found to produce fat infiltration after seven days, and no significant liver changes occurred in the controls. No reduction of food intake was found in mice fed aureomycin as compared to a group allowed to eat ad libitum.

Comparison studies of aureomycin, terramycin and chloramphenicol given orally and intraperitoneally revealed only slight changes in livers of animals receiving the first two drugs orally and none in those given chloramphenicol. When aureomycin and terramycin were administered intraperitoneally, however, marked infiltration occurred consistently with little or no change seen in the controls or in chloramphenicol-treated animals. The use of larger oral doses (400 mg. per kilogram or more) slightly increased the effect of aureomycin in producing fat vacuolization, but early death also occurred in a larger number of mice. Similar large doses of terramycin given intraperitoneally caused rapid death in the majority of animals, and no conclusions could be drawn. Again chloramphenicol administered in doses comparable to the other two antibiotics produced only mild changes. Heat-inactivated aureomycin (by incubation at 37° C. for eighteen hours) given subcutaneously produced liver changes similar to those in animals receiving active aureomycin.

These reports suggest that reversible fatty infiltration of the liver is a frequent result of the administration of aureomycin and terramycin in both humans and animals. Such changes also appear to be more common after large doses, particularly when given intravenously. A number of questions remain to be answered, however, before it can be concluded that such fat vacuolization so produced represents a toxic or damaging effect upon the liver. This is emphasized by the fact that beneficial effects of aureomycin in patients with liver disease have been reported (J. M. Shaffer, L. W. Bluemle, V. M. Sborov, and J. R. Neefe, *Am. J. Med. Sci.* **220,** *173 (1950)*), and aureomycin has also been found to be protective in rats receiving yeast diets which produce hepatic necrosis (P. György, J. Stokes, W. H. Smith, and H. Goldblatt, *Ibid.* **220,** *6 (1950)*). The possible mechanisms responsible for the appearance of fat in the liver during antibiotic administration still require elucidation. The relationship of change in bacterial flora to the hepatic changes, an obvious possibility in view of the known effect of these antibiotics, appears to be in doubt in view of the finding of Lepper *et al.* (*loc. cit.*) that inactivated aureomycin was as effective in producing the liver changes as was active aureomycin similarly

administered. Further investigation of the nutritional and metabolic effects of aureomycin and terramycin is needed before their use over prolonged periods or in large doses can be condemned, particularly in patients in need of antibacterial therapy. Hepatic function should, however, be observed closely in such patients.

MECHANISM OF TYROSINE OXIDATION

Earlier reports have indicated a role of ascorbic acid in the metabolism of the amino acid tyrosine. It has been observed that guinea pigs deficient in ascorbic acid excrete large quantities of homogentisic acid, para-hydroxyphenylpyruvic acid, and the corresponding lactic acid derivative in the urine when fed tyrosine. Premature infants excrete these same materials, and the quantities excreted decrease when supplements of ascorbic acid are provided (*Nutrition Reviews* **5,** *173 (1947)*). Subsequent reports provide evidence that folacin has a similar or additional role in the metabolism of tyrosine, since the hydroxyphenyluria of scorbutic guinea pigs and of premature infants decreases with provision of folacin without ascorbic acid (*Ibid.* **8,** *260 (1950)*).

It has been observed that tyrosine oxidation is accomplished *in vitro* by concentrated suspensions of liver (R. R. Sealock and R. L. Goodland, *J. Biol. Chem.* **178,** *939 (1949)*), and that ascorbic acid added to these systems increases their activity (H. A. Painter and S. S. Zilva, *Biochem. J.* **46,** *542 (1950)*). The reaction utilizes four atoms of oxygen and produces one molecule of carbon dioxide and acetoacetate but no ammonia. It has not been possible to designate, or with uniformity to isolate, specific intermediate products in the *in vitro* oxidation of this amino acid. From observations in intact animals it has been postulated but not definitively demonstrated that para-hydroxyphenylpyruvate or 2,5-dihydroxyphenylalanine serves as a first intermediate, the reaction then proceeding through 2,5-dihydroxyphenylpyruvate to homogentisic acid and thence to acetoacetate.

Important contributions to the understanding of the oxidation of tyrosine and the role of ascorbic acid in this reaction have come recently from W. E. Knox and M. LeMay-Knox at the University of Cambridge (*Biochem. J.* **48,** *xxii (1951)*; **49,** *686 (1951)*). These workers have found that a transamination reaction limits the rate of oxidation of tyrosine in liver homogenates. By adding D-amino acid oxidase plus D,L-alanine the reaction rate was tripled. The same effect was observed when alpha-ketoglutarate was added, with production of stoichiometric amounts of L-glutamate. The initial reaction in the oxidation of tyrosine was thus shown to be a tyrosine-alpha-ketoglutarate transamination with formation of para-hydroxyphenylpyruvate. When the limitation of the over-all oxidative reaction was removed by addition of ketoglutarate, oxygen uptake approximated the theoretical value of four atoms per molecule of tyrosine with formation of one molecule of acetoacetate. Addition of ascorbic acid as well as alpha-ketoglutarate produced a further increase in the rate of reaction, a total increase of ten times in the oxidation of tyrosine. It was then possible to investigate the other intermediate steps in the oxidative metabolism of tyrosine and the role of ascorbic acid in the reaction.

The enzyme preparations in these studies were derived by homogenizing freshly removed rat livers in three parts of iced 0.9 per cent potassium chloride. Cellular and mitochondrial materials were removed by centrifuging at 6000 × g. for twenty minutes. The preparation was dialyzed against tap water. Activities of the enzyme solutions were retained for several days with storage in the refrigerator. Reactions were carried

out manometrically in 0.07 molar phosphate at a pH of 7.5.

In a further study of transamination as the initial step in oxidation of tyrosine by the liver enzyme it was shown that transamination is an obligatory reaction proceeding at a linear rate proportional to the concentration of enzyme. Transamination occurred at a rate exceeding that of the over-all oxidation of tyrosine. Partial removal of a coenzyme by dialysis decreased the rate of transamination and by this means limited the rate of aerobic oxidation of tyrosine. When boiled fresh enzyme or pyridoxal phosphate was added to the dialyzed system, the rate of transamination and of tyrosine oxidation increased. The products of the tyrosine-alpha-ketoglutarate transamination reaction were shown to be glutamate and para-hydroxyphenylpyruvate. The latter compound was determined by absorption in the ultraviolet at 320 millimicrons and by isolation of the 2,4-dinitrophenylhydrazone.

Addition of ascorbic acid to the reaction mixture was without effect on the rate of deamination of tyrosine, but the evidence achieved indicated that the vitamin was required for oxidation of para-hydroxyphenylpyruvate, the product of transamination. The catalytic effect was great enough in some experiments so that one mole of ascorbic acid permitted oxidation of 50 moles of para-hydroxyphenylpyruvate. This action of ascorbic acid seemed to be largely one of prolonging the oxidation of tyrosine to acetoacetate. Isoascorbic acid replaced ascorbic acid with similar catalytic effect. Other reducing agents, *e.g.*, glutathione, cysteine, and dihydroxymaleic acid, were inactive. Although folacin had been reported to restore tyrosine metabolism in scorbutic guinea pigs (C. W. Woodruff and W. J. Darby, *J. Biol. Chem.* **172,** *851 (1948)*), it had no effect on the catalysis of tyrosine oxidation in the liver enzyme system.

Knox and LeMay-Knox (*loc. cit.*), using the liver enzyme system, sought evidence of other products intermediate between para-hydroxyphenylpyruvate and acetoacetate in the oxidation of tyrosine. Their observations indicate two additional intermediate products. These occur successively in the oxidative reaction and are 2,5-dihydroxyphenylpyruvic acid and homogentisic acid. These compounds when provided as substrates in the enzyme system were oxidized to acetoacetate with the theoretical oxygen uptake. It was possible to inactivate the enzyme responsible for oxidizing homogentisic acid to acetoacetate. Under these circumstances formation of acetoacetate from tyrosine was stopped and a product identified as homogentisic acid accumulated in the reaction mixture. It is of interest that the system seems to possess a relatively high degree of specificity. Other substrates of related compounds were not oxidized. Those tested included tyramine, L-2,5-dihydroxyphenylpyruvic acid and gentisic acid.

These experiments, which were admirably conceived and carried out, provide important new information concerning the metabolism of tyrosine in the liver and on the role of ascorbic acid in the oxidative breakdown of this amino acid. To recount, it has been shown that L-tyrosine by transamination with alpha-ketoglutarate forms glutamate and para-hydroxyphenylpyruvic acid. Further oxidation intermediates are 2,5-dihydroxyphenylpyruvate and homogentisic acid. Finally, acetoacetate is formed by oxidation of homogentisic acid. Ascorbic acid catalyzes the reaction by an increased oxidation of para-hydroxyphenylpyruvic acid, although the actual point of action is not yet revealed. It is of interest that this keto acid accumulates in scorbutic guinea pigs fed tyrosine, providing evidence for the same mechanism in the intact animal.

The authors reason that this function of ascorbic acid in tyrosine metabolism may represent one of its functions as a vitamin. They point out that the quantity of ascorbic acid required to assure complete metabolism of tyrosine in the intact animal is related to the amount of tyrosine administered.

LYXOFLAVIN

In 1949 E. S. Pallares and H. M. Garza (*Arch. Biochem.* **22,** *63 (1949)*) described the isolation of L-lyxoflavin from human heart muscle. The yield was small—5 mg. from 10 kg. of myocardium—and it appeared that the lyxose earlier prepared from the same source was originally present as the flavin. This material was later synthesized, and a comparison of the per cent of nitrogen and the melting point of the tetra-acetates from both sources indicated that the natural and synthetic lyxoflavins are identical.

Lyxoflavin differs from riboflavin only in the nature of the carbohydrate component, which in the latter is D-ribose and in the former L-lyxose. Inasmuch as the final difference between the two compounds resides in the configuration around the C-4 of the pentose group, the possibility that lyxoflavin might exhibit vitamin action naturally arises. This possibility is enhanced by the demonstration that one unit of the vitamin B_{12} molecule is a derivative containing a 1,2-diamino-4,5-dimethylbenzene part linked through a nitrogen atom to a pentose, which orientation is common to both riboflavin and lyxoflavin (N. G. Brink *et al.*, *J. Am. Chem. Soc.* **72,** *1866 (1950)*).

In order to evaluate the biologic potency of synthetic lyxoflavin, G. A. Emerson and K. Folkers (*J. Am. Chem. Soc.* **73,** *5383 (1951)*) employed the conventional type of rat assay for riboflavin in which the latter was replaced by lyxoflavin. Even in doses of five to ten times that of the riboflavin which gave a growth response, lyxoflavin produced no more increase in body weight than was secured when the riboflavin-deficient ration was given alone. The lyxoflavin was then examined by microbiologic assay using *Lactobacillus casei* as the test organism. In this test it showed less than 0.2 per cent of the activity of riboflavin under similar experimental conditions.

An experimental ration for rat assay for unidentified vitamins was then devised; the protein of this ration was supplied by a diet containing 60 per cent soybean meal, 4 per cent salt mixture, 24 per cent dextrose, 10 per cent vegetable shortening, 2 per cent cod liver oil, and the various recognized vitamins. In order to exaggerate the metabolic need and thus enhance the development of the state of possible deficiency, 0.5 per cent of desiccated thyroid was added to the basal ration. The animals were restricted to this diet for twenty-eight days after weaning, when they were grouped according to body weights and each group was given one of a number of natural food materials to be examined for possible unidentified vitamin activity. It was found, under these conditions, that defatted liver powder (Viobin), menhaden fish meal, Wilson's liver fraction L, and water-insoluble liver solids, each fed at a 10 per cent level, stimulated growth above that on the basal diet. In two different experiments in which lyxoflavin was added (150 micrograms per day), there was unmistakable evidence of growth promotion of the same order of magnitude as that produced by the natural source materials.

Inasmuch as riboflavin is recognized as an indispensable factor for nutritive success in man, it would be interesting to test lyxoflavin on human subjects. Prior toxicologic tests were carried out on mice; lyxoflavin in doses some two thousand times the daily requirement of this species for riboflavin was given for thirty days with no detectable difference between the controls and the treated mice. Subsequent preliminary tests on patients with hypertension showed no effect on temperature, pulse, respiration, urine, or blood.

From this recent work it appears that there exist compounds with vitamin activity which are yet unidentified, and that lyxoflavin may be one of these.

NOTES

Letter to the Editor

Dear Sir:

Your review "The Febrile Response to Intravenous Fat" of June 1951 (*Nutrition Reviews* **9,** *163 (1951)*) refers extensively to our paper of the same title (G. F. Lambert, J. P. Miller, and D. V. Frost, *Am. J. Physiol.* **164,** *490 (1951)*), and to papers by H. C. Meng (*J. Lab. Clin. Med.* **37,** *222 (1951)*), and E. M. Neptune, R. P. Geyer, I. M. Saslaw, and F. J. Stare (*Surg. Gynec. Obstet.* **92,** *365 (1951)*). These are the only three groups we are cognizant of that have published information regarding the testing of intravenous fat emulsions for pyrogenicity in rabbits. The interpretation of your review is that our emulsions were pyrogenic whereas those studied by the other groups were nonpyrogenic. A careful comparison of Meng's data with ours suggests very little actual difference. No direct comparison can be made between the findings of Neptune's group and ours because of the lack of published data.

According to your review, Meng's data indicate that his fat emulsions were nonpyrogenic in rabbits. It may be pointed out, however, that we used the U.S.P. XIII pyrogen test while Meng did not. Because we stated that our emulsions failed to pass the U.S.P. test, but did not present the individual data, it was impossible for the reviewer to make a direct comparison between our results and those of Meng. The U.S.P. test specifies that if 5 rabbits are used (dose: 10 ml. per kilogram), a temperature increase of 0.6°C. or more in 2 or more of the group constitutes a positive test for pyrogens. Meng reported the temperature rise as the average for 5 rabbits, *i.e.*, +0.55°C. for ten-day-old emulsions and +0.67°C. for one-month-old emulsions. Temperature rises of nine of our freshly prepared emulsions calculated in an identical manner yield averages of +0.33, +0.60, +0.06, +0.47, +0.32, +0.48, +0.18, +0.32, and +0.48°C. Eight of these nine emulsions gave average temperature rises that were less than those reported for Meng's emulsions; however, none of the nine fulfilled the specifications of a nonpyrogenic solution as tested by the U.S.P. pyrogen test. Thus Meng's emulsions were apparently no more "nonpyrogenic" than ours.

The Neptune paper is primarily concerned with clinical findings. It does indicate, however, that all emulsions used were first tested in rabbits and found to be nonpyrogenic. Whether the U.S.P. method was used is not stated. Thus accurate comparison with our results, or with those of Meng, is not possible.

The review states: "In the work of Lambert *et al.*, only bacterial pyrogens seem to have been ruled out." Actually the discovery that something other than bacterial pyrogen is capable of eliciting the pyrogen response in rabbits came as a very real surprise. The primary object of our work was then to establish to the general satisfaction that the febrile response to our experimental fat emulsions was not caused by bacterial pyrogen. The nature of the other thermogenic substance(s) is not yet established. This situation, therefore, poses one of the problems incidental to the development of clinical fat emulsions in general.

The hypothesis of our paper that fat emulsions are inherently pyrogenic is certainly open to criticism. The idea that the temperature rise might be caused by excessive heat produced by the rapid oxidation of fat was not original with us, but was advocated as one attractive hypothesis. We were careful, however, to disclaim any real evidence for this hypothesis, pointing out that other explanations are just as tenable. One such explanation considered in our paper and in your review concerns oxidation products of fats.

The fact that the U.S.P. pyrogen test does not differentiate between bacterial

pyrogen and substances which may occur in fat emulsions has significance from many aspects. A method was suggested by us to test for absence of bacterial pyrogen by breaking the emulsion and testing the aqueous phase. Before fat emulsions become available commercially, further attention will have to be given to the establishment of suitable standards. We do not yet know whether the unknown pyrogenic substances in certain fat emulsions will cause disturbances in humans.

It was certainly not the intention of any part of our paper to discourage further work on fat emulsions, but it was merely an attempt to define what appeared to us to be a common problem. We regret this interpretation by the reviewer, but at the same time take exception to his view that the problems to be overcome in the manufacture of fat emulsions are purely technical ones. There appear to us to be certain problems both technical and physiologic which require clear definition and intensive research.

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Teaching Nutrition on a Global Scale

The September 1951 issue (*Nutrition Reviews* **9,** *287* (*1951*)) contained a review of a booklet written by the Food and Agriculture Organization of the United Nations entitled "Teaching Better Nutrition—A Study of Approaches and Techniques." This interesting booklet is now available in both Spanish and French editions from FAO, Viale delle Terme di Caracalla, Rome, Italy.

Nutrition Surveys

The methods and pitfalls in conducting nutrition surveys have been repeatedly discussed in these columns (*e.g.*, *Nutrition Reviews* **8,** *111*, *114* (*1950*)). Some new points of view have been presented by H. M. Sinclair (*New Engl. J. Med.* **245,** *39* (*1951*)), and in a recent editorial in the *Lancet* (**II,** *390* (*1951*)).

Sinclair first recalled some of the ancient history of nutrition investigation and disturbances of the nutrition of populations. He then discussed in some detail the methods used in nutrition surveys, especially those of the Oxford Nutrition Survey, which was organized in 1941 under the auspices of the Ministry of Health in England. He emphasized the place of nutrition surveys both in the university and in public health. He pointed out that the Ministry of Health needs to be well informed of the nutritional status of the population it is responsible for.

The editorial in *Lancet* first reviewed briefly the works of A. Keys *et al.* ("The Biology of Human Starvation," *Univ. of Minnesota Press, Minneapolis* (*1950*)). The important results of the Cambridge nutrition team under the auspices of Professor R. A. McCance were then outlined, particularly the survey unit set up in Wuppertal. Because of the large quantity of negative biochemical examinations obtained by this group in a population known to be undernourished and often hungry, the editorial discourages the hope that the biochemical laboratory can give valuable assistance in assessing the nutritional status of a population.

Surely the shortcomings of the biochemical laboratories need not discourage one from continuing to use these laboratory facilities and particularly to refine them so that they can be of aid in evaluating the nutrition of an individual or of a population group. It certainly can be agreed with the editors of *Lancet* that sound clinical judgment and wide social experience are necessary for the proper conduct of a nutrition survey.